Ph.D Synopsis 2021-22

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DATE OF R.A.C.	**27/07/2021**
DATE OF R.R.C.	**05/04/2022**
PLACE OF R.R.C.	**S.R.T.M UNIVERSITY, NANDED.**

"FORMULATION, DEVELOPMENT AND EVALUATION OF NANOCARRIERS TARGETING FOR RHEUMATIC ARTHRITIS"

A SYNOPSIS SUBMITTED FOR THE REGISTRATION OF *DEGREE OF*

DOCTOR OF PHILOSOPHY

In the Faculty of Pharmaceutical Sciences & Technology

Submitted

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(2021-2022)

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1. Introduction

One of the most conventional drug delivery is by oral route of administration. For oral absorption of drugs water solubility is most important. Most of the drugs discovered today having poor water solubility thereby affecting bioavailability of orally administered drugs. Also, it is reported that approx. 40% of currently marketed drug and up to 70% of newly developed drugs are poorly water soluble[1]. Major rate limiting step in the absorption of drug is its poor solubility and thereby reducing bioavailability. When an active agent is given orally, it must first dissolve in gastric and/or intestinal fluids before it can permeate the membranes of the GI tract to reach systemic circulation. Therefore, a drug with poor aqueous solubility will typically exhibit dissolution rate limited absorption, and a drug with poor membrane permeability will typically exhibit permeation rate limited absorption. Hence, two areas focus on improving the oral bioavailability of active agents include:

1. Enhancing solubility and dissolution rate of poorly water-soluble drugs.
2. Enhancing permeability of poorly permeable drugs.

Among other challenges of conventional drug delivery are like luminal degradation, pre-systemic metabolism, poor drug stability, efflux mechanism, therapeutic effectiveness, targeted delivery of drug, generalized side effects etc.

Many novel approaches can be used to overcome these challenges such as Micronization, Nanonisation, Solid lipid nanoparticle, Liposomes, Nanostructured lipid carriers (NLC) etc

Nanotechnology offers numerous advantaged over conventional drug delivery stability, biocompatibility, enhanced permeability, retention effect, controlled release of drug that can be triggered by pH, redox potential, presence of certain enzyme, temperature etc. It also offers functionalisation of nanomaterials means binding to specific ligand, antigen, protein etc on surface of nanomaterials and increase their accumulation in tissue, in tumour region or organ inflamed area. Nanomaterials can bypass biological barriers like blood brain barrier and allowing delivery of high drug concentration in target tissue.

Nanotechnology increases oral bioavailability of drugs due to their specialized uptake mechanisms such as absorptive endocytosis[2].

Nanomaterials are materials sized in dimension between 1& 100nm.Many of the current "nano" drug delivery systems at the scale of 100 nm or less in the nanometer range are such as liposomes, polymeric micelles, nanoparticles, dendrimers, and nanocrystals, metallic nanoparticle, quantum dots, colloidal gold particles in nanometer sizes[3]. Being nanosized, these structures penetrate in the tissue system, facilitate easy uptake of the drug by cells, permit an efficient drug delivery, and ensure action at the targeted location[4].

Nanomaterial based immunotherapy represent novel approach for cancer treatment, auto immune diseases, and regenerative medicine therapies. Autoimmune diseases are group of chronic diseases that arise from inappropriate immune response against self antigen resulting in inflammation & destruction of healthy tissues[5]. Nanocarriers or nanomaterials that are either lipid based or polymer based can be used to deliver anti-inflammatory molecule in target tissues.

Over 80 different auto immune diseases are there can affect almost any part of the body including brain, nerves, heart, muscle, skin, eyes, joints, lungs, glands, digestive tract, blood vessels, kidney. Rheumatoid arthritis is one of autoimmune disease affecting bones and muscles.

Rheumatoid Arthritis is a complex multifactorial joint associated autoimmune heterogenous group of inflammatory disorder. RA is characterized by an inflammation of the synovium with increased production of proinflammatory cytokines (IL-1, IL-6, IL-8, and IL-10) and by the destruction of the articular cartilage and bone, and it is associated with the development of cardiovascular disorders such as heart attack and stroke[6].

Treatment options for RA sufferers, the therapy is often associated with severe adverse effects and is ineffective in a large segment of the patient population. Due to the serious side effects of traditional RA treatment strategies, and even ineffectiveness for some patients, it is urgent to seek new treatment protocols. The main benefit of employing nanocarriers or nanoformulations in the therapeutics arena is to achieve targeted delivery using the optimum drug dosage, extended drug circulation, reduce side effects, and decrease the likelihood of developing drug resistance[7].

Benefit from intra-articular delivery of therapeutic Nano-formulations on multiple levels including: improvement in drug efficacy, reduced side effects due to the containment of

drugs in the joint space. The main challenge in intra-articular drug delivery is to prolong the retention time of drugs within the synovial joint. The versatile nature of the different nanoscale carriers makes them highly suitable for targeting and retaining drugs at different loci of the synovial joint[8].

In general, the agents for RA treatment are divided into non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (GCs), disease-modifying anti-rheumatic drugs (DMARDs), and biological agents. Due to the long-term and progressive nature of RA, patients must tolerate pain caused by the continued/repeated medication and suffer the adverse reactions caused by the distribution of the drug system. It has progressive joint destruction and extra-articular systemic manifestations. Studies show that 50–70% of patients lose their ability to work after 10–15 years of the disease development, which not only brings mental and economic burden to patients and their families, but also drags social development. Despite conventional RA treatment having achieved certain results, treatment tolerance and high-risk organ failure still exist. Therefore, it is still highly urgent to develop new treatment strategies for RA therapy[9].

2. Need of study:

RA affects approximately 1.0% of the population in developed countries, affecting women three-times more than men.

Conventional RA drug delivery limitations include non-specific biodistribution, low bioavailability and/or short half-life into the body. Moreover, high dosages are needed to be administered to patients, which cause off-target side effects.

"Direct drug delivery to bone and cartilage in RA can be achieved by using nanotechnology and another approach involves applying nanoparticles (NP's) in tissue engineering. NP's better penetration and accumulation at inflamed joints, retention of drug in cartilage and within synovium improving its therapeutic efficacy. Also prevents non-specific absorption by other organs and fast clearance from the joint cavity."[10]

Newly emerging area of Nanotechnology offers great advantages to improve solubility, permeability and direct targeted action of drug at desired site with controlled and sustained delivery of formulation.

Pathophysiology of rheumatic arthritis suggests the presence of macrophages and over expressed molecules within inflamed tissue, these increases the permeability so nanomedicines can accumulate in inflamed tissue and retention phenomenon, or be surface conjugated with a ligand to actively bind to receptors overexpressed by cells within chronic inflammatory tissues, leading to increased efficacy and reduced systemic side-effects.

Attempts will be made in present study to formulate nanomaterials that will be helpful to improve solubility as well targeting delivery of antiarthritic drugs exclusively to inflamed joints, thereby avoiding potential side effects associated with conventional RA therapy.

Some of the attractive features for an ideal drug delivery system would be high loading capacity, high encapsulation efficiency, ease of operation and cost-effectiveness, either for immediate or extended release, prime being easy modulation of drug release profile depending upon the properties of polymer/polymeric blends/other materials used.

3. Hypothesis:

Nanomedicines can be made use to target rheumatic arthritis by inflammatory tissues as target for drug delivery,[11,12]taking advantage of enhanced permeability, increasing site specific delivery by active targeting. Development of biocompatible nanomaterials and delivery systems for current antirheumatic agents may ultimately lead to lower effective doses, reduced dose frequency and more effective therapies with less systemic side-effects.[13]

4. Review of literature:

Payab et al. Triamcinolone acetonide (TA) is a synthetic corticosteroid with a potent anti-inflammatory effect. It is administered as an injectable suspension for the treatment of different inflammatory conditions including arthritic disorders, macular edema and diabetic retinopathy. In the present work, TA-Eudragit® RS100 nanofibers and nanobeads were formulated and the physicochemical characteristics of the nanoformulations were assessed.

A custom-designed electrospraying system was used for preparation of the TA–Eudragit® RS100 electrosprayed samples.

Ansari et al. prepared Zinc gluconate loaded chitosan nanoparticles using chitosan and sodium tripolyphosphate in double-distilled water, and zinc gluconate was simultaneously added along with the synthesis of chitosan nanoparticles. Nanoparticles were characterized for various physicochemical properties, and then anti-arthritic potential was investigated against collagen-induced arthritis in Wistar rats.They observed that the treatment of rats with both zinc gluconate and zinc gluconate loaded chitosan nanoparticles reduced the severity of arthritis by reducing joint swelling, erythema, and edema but zinc gluconate loaded nanoparticles exhibited superior efficacy.

Bacelo et al. The main objective of this work was to develop an implantable system capable of capturing excessive TNF-alpha present in intra-articular cavities of an RA patient. For that, immobilized a neutralizing TNF-α antibody at the surface of a polymeric substrate (i.e., electrospun nanofibers). This strategy takes advantage of specific interactions between the antibody and the antigen, where the antibody (TNF-α antibody) binds to the antigen (TNF-α) avoiding/limiting its harmful pro-inflammatory effects.

Singh et al. The aim of the present investigation was to prepare the polymeric nanofiber composite of vitamin B12, HA(hyaluronic acid_, and PVA as a formulation component for sublingual delivery of nicorandil. HA, being a component of the extracellular matrix, serves as a natural supplement for damaged tissue and facilitates quick recovery from a local injury and inflammation. Evidence further suggests that HA reduces the amount of

pro-inflammatory cytotoxins, thereby showing its beneficial effects on inflammatory arthritis. In addition, the viscoelastic properties of HA ensure controlled release of entrapped drug with a prolonged retention time at the site of application

He, et al. fabricated electrospun gelatin/polycaprolactone (GT/PCL) nanofibrous membranes, and found that acetic acid could improve the miscibility, contributing to obtaining finer and compositionally homogeneous hybrid nanofibers. GT/PCL membranes were fabricated by electrospinning. In brief, polymers of gelatin type A (300 Bloom) from porcine skin in powder form and PCL (MW, 80,000) were used.

Shan et al. In this study, the different encapsulation methods involving emulsification and coaxial electrospinning were both utilized to fabricate a series of core/sheath composite, nanoparticles (NPs) and Nanofibers (NFs) separately, for drug delivery. Bovine serum albumin (BSA) was employed as an active pharmaceutical ingredient model for core; poly(L-lactic acid) (PLLA) and methoxy poly(ethyleneglycol)-Poly lactic acid (mPEG PLA) were selected as the encapsulation matrix for sheath.
NPs containing model drug of BSA were formulated using a modified multiple emulsion–solvent evaporation technique NFs containing model drug of BSA were fabricated by coaxial electrospinning.

Sharma and Chaudhary present investigation was undertaken to prepare bromelain loaded nanostructured lipid carriers (Br-NCs) with high drug loading, stability and efficacy in rheumatoid arthritis management. Br-NCs fabricated via double emulsion solvent evaporation method were characterized for physical properties, morphology and stability. The protective potential of orally administered Br-NCs in rheumatoid arthritis was evaluated via assessing arthritis severity in wistar rats along with biochemical, hematological and immunological parameters. Br-NCs remarkably ($p < 0.05$) diminished paw edema, joint stiffness, mechanical allodynia and tissue damage along with alleviation of oxidative stress and immunological markers.

Webber et al. report here on the preparation of a modular peptide amphiphile (PA) capable of releasing an anti-inflammatory drug, dexamethasone (Dex), by conjugation via a labile hydrazone linkage. This molecule self-assembled in water into long supramolecular nanofibers when mixed with a similar PA lacking the drug conjugate, and the addition of calcium salt to screen electrostatic repulsion between nanofibers promoted gel formation. These nanofiber gels demonstrated sustained release of soluble Dex for over one month in physiologic media. The Dex released from these gels maintained its anti-inflammatory activity when evaluated in vitro using a human inflammatory reporter cell line.

Williams et al. Naringenin (NAR) is recognized for its anti-inflammatory activity. Study aimed to improve the therapeutic efficacy of NAR by formulating it into nanocrystals (NCs) via wet milling. The obtained NARNCs exhibited superior dissolution behaviors, increased cellular uptake, and enhanced transcellular diffusion relative to those of bulk NAR. Oral administration of NARNCs also significantly improved bioavailability in rats. In addition, the NARNCs effectively improved rheumatoid arthritis treatment in collagen-induced arthritic rats by reducing inflammatory cell infiltration and synovial damage.

Chaudhary et al. The study aims to develop a nanoparticles gel containing novel natural polymer-based methotrexate nanoparticles and evaluate its therapeutic potential on Complete Freund'sAdjuvant– Induced Arthritis rat model. Optimized nanoparticle gel formulation F3W2 can be effective in the treatment of rheumatoid arthritis.

Arslan et al. a nanofiber-hyaluronic acid membrane system that was prepared by using a quarter of the concentration of commercially available hyaluronic acid supplement, Hyalgan®, was used for the treatment of an osteoarthritis model, and Synvisc®, which is another commercially available hyaluronic acid containing viscoelastic supplement, was used as a control. The results show that this system provides efficient protection of arthritic cartilage tissue through the preservation of cartilage morphology with reduced osteophyte formation, protection of the subchondral region from deterioration, and maintenance of cartilage specific matrix proteins in vivo. This work demonstrates for the

first time that this hybrid nanofiber membrane scaffold can be utilized as a potential candidate for osteoarthritis treatment.

Yan et al. Glucocorticoids (GCs) show powerful treatment effect on rheumatoid arthritis (RA). Here established a biomimetic exosome (Exo) encapsulating dexamethasone sodium phosphate (Dex) nanoparticle (Exo/Dex), whose surface was modified with folic acid (FA)-polyethylene glycol (PEG)-cholesterol (Chol) compound to attain FPC-Exo/Dex active targeting drug delivery system. The present study provides a promising strategy for using exosome as nanocarrier to enhance the therapeutic effect of GCs against RA.

Kim et al. The fibers are produced by electrospinning copolymers of N-isopropylacrylamide (NIPAAm) and N-hydroxymethylacrylamide (HMAAm). Dextran-containing NFs were prepared by electrospinning the copolymers blended with fluorescein isothiocyanate (FITC)-dextran, and the 'on–off' switchable release of FITC-dextran from the crosslinked NFs was observed.

Siddique et al. prepared celecoxib, gingerol and oleanic acid loaded PLGA nanoparticles by solvent evaporation method and nanoparticles were characterized by particle size, zeta potential, polydispersity index, entrapment efficiency and FTIR. FCA is induced in right hand paw of rats for induction of arthritis. Celecoxib, gingerol and oleanic acid loaded PLGA nanoparticles coated with chitosan were given orally to rats for the evaluation of anti-arthritic effect of this nanoformulations in rats. Double emulsion method was used to prepare drugs (celecoxib, oleanic acid, gingerol) loaded with PLGA nanoparticles. At the end of study, it was concluded that PLGA loaded celecoxib, gingerol and oleanic acid coated with chitosan have excellent effects in minimizing the side effects and increasing the therapeutic efficacy of drugs.

Nimitha et al. In this study leflunomide loaded solid lipid nanoparticle subcutaneous injection was prepared, characterized and studied for various in-vitro, in-vivo evaluations. It has been observed that solid lipid nanoparticle can encapsulate considerable amount of

leflunomide with smaller particle size and shape, which is suitable for subcutaneous injection. In-vitro and pharmacokinetic studies proved its prolonged action, there by solid lipid nanoparticle system can be used as drug carrier for lipophilic drugs. In-vivo studies proved that leflunomide loaded solid lipid nanoparticle have better antiarthritic activity.

Ha et al. The aim of this study is to design photothermally controlled drug release from multifunctional nanoparticles (MNPs) at a near-infrared (NIR) irradiated site to improve therapeutic efficacy for RA and reduce side effects. Au film was deposited onto methotrexate (MTX)-loaded poly(ethylene glycol)-poly(lactic-co-glycolic acid) (PLGA) nanoparticles, resulting in MTX-loaded MNPs. The synergistic effects of MTX-loaded MNPs with NIR irradiation were investigated using RA fibroblast-like synoviocytes (FLSs) and collagen-induced arthritis (CIA) mice.

5. Objectives:

Present study aimed for, Formulation, Development and Evaluation of Nanocarriers Targeting for Rheumatic Arthritis

With objectives-

- To formulate nanocarrier by using QbD approach.
- To study compatibility of drug and excipients by analytical techniques.
- To characterize formulated dosage form.
- To study stability parameters as per ICH guidelines.
- To study *in vitro* dissolution study.
- To perform *in vivo* evaluation study.

6. Plan of work:

- Literature survey.
- Selection of drug, procurement of drug and excipients.
- Authentication and solubility determination of selecteddrug.
- Formulation of desired dosage form like nanoparticles, nanofiber &/or phytosome, by QbD approach.
- Characterizations of formulation by

    a. Yield and drug content
    b. Drug loading efficiency
    c. Particle size and zeta potential
    d. Encapsulation efficiency
    e. Fourier transform infrared spectroscopy
    f. XRD study
    g. Differential scanning colorimetry (DSC)
    h. Scanning Electron Microscopic Analysis (SEM)
    i. In vitro drug release

- Evaluation of formulation
    - *In vitro* anti-inflammatory activity.
    - *In vivo* anti-inflammatory (Anti-arthritic activity) study.
- Stability study as per ICH guidelines.

7. Material & Methods:

1. Literature review

2. Selection and Collection of antirheumatic drug & excipients

Selection of antirheumaticdrug: e.g.,NSAID, glucocorticoid, disease modifying anti-rheumatic drugs, biological agents or herbal extract.

Selection of a polymer: To formulate the nanocarrier, suitablepolymer will be selected.

3. Experimental work

A. Preformulation Studies

1. Spectroscopic study
2. Solubility study
3. Crystallinity study

B. Compatibility study

4. Formulation development

Preparation of drug loaded nanocarrier such as nanoparticles, nanofibers may be loaded with synthetic drug molecules or herbal extract (phytoconstituents) &/or phytosome as nanocarriers may be prepared.

Formulation of dosage form may be done by few of the following methods:

A. Method of preparation of Nanoparticles[30]:

a. Solvent evaporation method
b. Spontaneous emulsification method
c. Salting out/emulsion diffusion method
d. Nonaqueous phase separation method
e. Nanoparticle Preparation by the Emulsion Polymerization Method

B. Method of preparation of Nanofibers [31]

In the simplest electrospinning experiment, termed monoaxial electrospinning, a solution of a polymer and drug is dispensed through a single-bore blunt-end needle, this gives a monolithic product, with the drug and polymer typically evenly and homogeneously blended throughout the fibres. It is possible to process two, three or even more liquids as compound jets (co-flowing liquid jets).

C. Method of preparation of Phytosome:[32]

Phytosomes are formulated by incorporating the standardized plant extract or the phytoactive compounds into phospholipids, mainly phosphatidylcholine (PC), to generate lipid-compatible molecular structures.

5. Characterization of drug-loaded Nanocarrier

1. Yield and drug content
2. Particle size and zeta potential
3. Encapsulation efficiency
4. Fourier transform infrared spectroscopy
5. XRD study
6. Differential scanning colorimetry
7. In vitro drug release

6. Stability studies

As per ICH guidelines.

7. In vitro anti-inflammatory activity/antirheumatic study

Using suitable cell lines study of parameters like cellular uptake (therapeutic efficacy), cell viability (biocompatibility), cell permeability etc

8. In vivo antirheumatic study

Effect of formulation on inflammation biomarkers and biochemical indicators.RA markers for monitoring paw & knee swelling, body weight loss, limb redness, locomotion behaviour etc.

Importance of study/ Society application:

- **International status:**

  Nanotechnology offers great advantages in drug delivery to improve therapeutic efficiency in various diseases. Nanocarriers enhance biocompatibility, stability, enhanced permeability and retention effects. Also use can be made of targeting drug retention and accumulation in tumour region, in tissue or in organ inflamed area. Nanocarriers can be used to target inflamed synovial joint to deliver anti rheumatic drugs. Development of biocompatible nanomaterial and delivery system for antirheumatic agent may lead to lower effective doses, reduced dose frequency, and more effective therapies with less systemic side effects.

  Rheumatoid arthritis (RA) is an autoimmune disease with a worldwide prevalence of approximately 0.5% to 1% among adults. When RA is left uncontrolled, the RA patient may experience joint deterioration, severe disability, decreased quality of life, the onset of comorbidities and premature mortality. The potential comorbidities include but are not limited to cardiovascular disease (CVD), cancer (specifically lymphoma and lymphoproliferative diseases, lung cancer and melanoma), infections, depression and gastrointestinal disease. In US, rheumatoid arthritis is emerging as one of the most commonly diagnosed autoimmune disease and its prevalence rate is increasing at an alarming rate. About 1.5 million people are living with rheumatoid arthritis in US during year 2020.

  Adalimumab (Humira), etanercept (Enbrel) and infliximab (Remicade) are currently among top ten best selling drugs in USA.[13]

- **United States patent US 2006/0264443 A1(23/11/2006):** Treatment of Rheumatoid Arthritis
- **European patentEP3 190 124 A1 (12.07.2017):** Adalimumab for use in therapy of rheumatoid arthritis.

  National status: In India, approximately one percent of the population suffers from rheumatoid arthritis. Therefore, almost 10 lakh people suffer from this type of arthritis. Most people assume that arthritis is a disease that older people suffer from and younger population is safe from it. Arthritis may start early in the age of 40 years affecting youngers also.

  Indian patent: The Indian Patent Office (IPO) has passed an order on 3 September 2015 (Order) rejecting patent application no. 991/MUMNP/2003 (Patent Application) to Pfizer Products, Inc. (Pfizer) for rheumatoid arthritis drug 'Tofacitinib'.

Significance of study:

Rheumatoid arthritis is a complex disorder associated with synovial joint inflammation and irreversible damage to bone and cartilage. Most common symptoms are joint pain, physical impairment, reduced mobility, depression, sleep difficulties etc. It affects working ability and is the leading cause of chronic morbidity. Its prevalence is about 1% in adult population, leads to inability to participate in work and social activity. Rheumatoid arthritis seriously affects patients' quality of life. 50 to 70% patient loose their ability to work after 10years of disease development, creating social and economic burden on patients family. Nanotechnology based immunotherapy represent a novel approach for cancer treatment, auto-immune diseases & regenerative medicine therapies. Nanocarriers can be used to deliver drug molecules to target tissues. Nanomedicines can be made use to target rheumatic arthritis by inflammatory tissues as target for drug delivery,[11,12]taking advantage of enhanced permeability, increasing site specific delivery by active targeting. Development of biocompatible nanomaterials and delivery systems for current antirheumatic agents may ultimately lead to lower effective doses, reduced dose frequency and more effective therapies with less systemic side-effects.[13]

Proposed work Plan/ Formulation and Structure of Study:

Year-wise Plan of work and targets to be achieved:

Sr. No.	Work	Duration of Work
1.	Extensive Literature Survey	6 Months
2	Selection And Collection of Drug and Excipients	3 Months
3	Preformulation Studies	3 Months
4	Formulation Development and Optimization of Nanocarriers	8 Months
5	Evaluation Of Optimized Formulation	8 Months
6	Statistical Analysis, Data Interpretation and Conclusions	3 Months
7	Communication With Research Journals & Publishing Data	3 Months
8	Thesis writing	2 Months

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